THE ADAPTIVE PRODUCTION OF ENZYMES BY BACTERIA

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Wortmann was probably the first physiologist to describe in biochemical terms the mechanism by which living cells can adapt themselves to the utilization of certain foodstuffs (83). He observed that the cells of an unidentified bacterial species produced amylase when grown in media containing starch, whereas this enzyme failed to appear when the same culture was grown in media which did not contain this polysaccharide. In the same paper Wortmann also recognized that, on the contrary, yeast always produces invertase, whether or not sucrose is a constituent of the medium. Since then, the influence of the composition of the culture medium on the enzymatic activity of microbial cultures has been analyzed by many investigators and it became recognized that the production of a given enzyme might be greatly stimulated when the substrate which it attacks is a constituent of the culture medium. In the appended bibliography, an attempt has been made to present a fairly complete list of the publications dealing with this subject.

Karström designated as "adaptive" those enzymes which are produced as a specific response to the presence of the homologous substrate in the culture medium. He differentiated them from the "constitutive" enzymes which are always formed by the cells of a given species, irrespective of the composition of the medium (45, 48, 49).

Karström's terminology is convenient and has found wide acceptance. It is not sufficient, however, to describe fully the complex influence which the environmental factors, and in particular the composition of the culture medium, may exert on the

enzymatic constitution of the microbial cell. For instance, cultures of *Proteus vulgaris* grown in media containing *l*-leucine or *d*-isoleucine are very rich in urease; when grown in Uschinsky's synthetic medium, however, the same bacterial species forms only traces of urease although catalase is abundantly produced (43, 62). It is also known that calcium bears a definite relation to the formation of gelatinase by some bacterial species (34, 35, 56). In other words, the production of a microbial enzyme may be stimulated by the presence in the medium of substances unrelated to the substrate attacked by the enzyme (65, 66).

On the other hand, the stimulating effect of a given substrate upon the production of the homologous enzyme may exert itself through a number of different mechanisms. For instance, we may be dealing with a microbial culture capable of producing a variant endowed with a new enzymatic property. If the substrate attacked by this new enzyme is present in the medium, the variant may be favored to multiply rapidly, and the new enzyme will accumulate in the culture.

Escherichia coli-mutabile offers an example of this mechanism which has been carefully analyzed. This bacterial species usually does not produce the enzyme lactase; it becomes capable, however, of rapidly fermenting lactose after repeated transfers in lactose broth. Lewis has made the important observation that *Escherichia coli-mutabile* always produces a few cells (in a ratio of 1:1,000,000) capable of attacking lactose, even when grown in lactose-free media (53). It is obvious that the presence of lactose in the medium will favor the multiplication of these lactose-fermenting variants, and therefore stimulate the accumulation of lactase in the medium. Thus, many cases of "training" are undoubtedly due to a natural selection of a variant form. It is a characteristic of the new properties—a new enzymatic function, for instance—which are the results of natural selection, that they develop slowly and progressively in successive transfers of the culture in the specific medium which calls them forth. Once developed, however, the new property appears stable for a number of generations, even though the culture is transferred

repeatedly into media not containing the substrate with reference to which variation has taken place (36, 52, 55, 59, 62, 63, 64).

It is in this respect that the "adaptive" enzymes considered in the present review exhibit a distinctive, different behavior. They appear without delay when the cells of the proper microbial species start multiplying in a medium containing the specific substrate; the specific enzymatic activity reaches its maximum development during growth of the very first transfer into the specific medium, but the enzymes again fail to be formed as soon as the cultures are transferred to media lacking the specific substrates. An adaptive enzyme therefore appears as a specific response of the cell to the presence of a given substance in the medium; it does not conform to the behavior of hereditary variations.

MECHANISM OF FORMATION OF ADAPTIVE ENZYMES

It is well known that the enzymatic activity of the *intact* living cell is affected by a great many factors, such as the age of the culture, cellular permeability, etc. (2, 65, 66, 80, 81, 82). It is not possible, therefore, to determine quantitatively the concentration of a given enzyme present in a culture from the rate of the reaction observed when the living cells are used as source of enzyme. In other words, increase in enzymatic activity of a culture does not necessarily mean increased production of enzyme. In many instances, however, the presence of adaptive enzymes has been demonstrated by testing cells killed with toluol (46, 47), or filtered autolysates (9, 10, 12, 14) under conditions where factors of cellular permeability could not vitiate the interpretation of the results. It remains possible, however, that in other cases, an increase in enzymatic activity, or the appearance of a new enzymatic function may be due, not to the production of a new enzyme, but to other mechanisms which result in the activation of enzyme systems already present (61).

Moreover, it is likely that in many, if not all cases, the adaptive response may take the form of an increased production of the specific enzyme, rather than the formation of a new enzyme.

Euler, for instance, found that invertase is produced by *Escherichia coli* on many media not containing sucrose, but that the production of this enzyme is increased many times (40 times in some cases) when sucrose is a component of the medium (18, 19, 21 to 26).

As stated earlier in this review, adaptive enzymes are characterized by the fact that they appear and reach their maximum activity during growth of the first transfer of the culture in the specific medium, and fail to be formed as soon as the culture is again transferred into a medium not containing the specific substrate. It appears unlikely that a character acquired and lost so suddenly could be due to natural selection of variant forms endowed with the new enzymatic property. Against the natural selection hypothesis is also the fact that in many cases the newly acquired enzyme can hardly be of any value to the organism which produces it. Yudkin, for instance, offers as an illustration the adaptive production of hydrogenlyase by *Escherichia coli* grown in a formate medium (84, 85, 86). The reaction $HCOOH = H_2 + CO_2$ which is catalysed by hydrogenlyase can at best liberate only very small amounts of free energy, and it is unlikely that the products of the reaction are required by the organism for its growth. He remarks also that, on the basis of the natural selection hypothesis, the immediate loss of the hydrogenlyase when the organism is transferred to a medium deficient in formic acid would suggest that the loss of the enzyme is an advantage to the cells grown in plain broth, an unlikely assumption. From these considerations, Yudkin decides that the production of hydrogenlyase can not be due to natural selection.

The conversion of creatine into creatinine by an adaptive enzyme is another example of a reaction which appears to be of little use to the organism involved (14). Even more striking, however, is the fact that the enzyme is formed equally well when creatinine (the end product of the reaction) instead of creatine is added to the medium. This observation is of some theoretical significance.

It is obvious that the natural selection hypothesis would be ruled out if the production of adaptive enzymes could be obtained

in the absence of any cellular division. Stephenson and Stickland working with hydrogenlyase (71, 72, 73) and Stephenson and Yudkin (74) working with yeast galactozymase, claim to have established this fact. Their conclusion is based on the following evidence: a) washed cells not possessing the enzyme were resuspended in a solution of the specific substrate, and enzyme formation could be demonstrated within one hour, *i.e.*, in a time too short for appreciable cell division to take place; b) on the basis of viable and total cell counts during the adaptation, it was found that enzyme production occurred without increase in cell numbers (the results were considered accurate within 5 per cent).

These observations suggest strongly that enzyme production can occur in the absence of cell multiplication; they do not, however, mean that enzyme production can occur without the synthesis of new protoplasm. It is a fact that most bacterial cells, when transferred to a new medium, undergo a phase of enlargement and elongation prior to cell division (40, 41, 68, 79). During this period, the metabolism of each individual cell increases, a phenomenon probably associated with the production of new protoplasm not accompanied by cell division. It must be mentioned at this point that, in spite of many attempts by several investigators, it has been found impossible to observe the production of enzymes in the presence of protoplasmic poisons or under conditions incompatible with cell growth (9, 10, 20, 74). Moreover, even in the case of formic hydrogenlyase which was produced in the absence of cellular multiplication, Stephenson and Stickland (72, 73) observed that no enzyme was formed unless some bouillon was added to the formate solution. It seems, therefore, that although the adaptive production of enzymes can occur in the absence of cellular division, it always involves the synthesis of new protoplasm. Hegarty has shown that physiologically young cells adapt themselves much more rapidly than older cells (38).

Some authors have tried to describe the mechanism of enzyme production in chemical terms. Quastel (65, 66) showed that, although the production of catalase, urease and fumarase by

Micrococcus lysodeikticus varies greatly according to the medium in which the organism is grown, the presence of urea does not stimulate urease, nor does that of succinate or fumarate stimulate fumarase production. It will be recalled also that the production of certain enzymes (urease and gelatinase) is conditioned by the presence in the culture medium of substances entirely unrelated to the homologous substrate (34, 35, 43, 56, 62, 65, 66). All these results, according to Quastel, are best interpreted by assuming that the enzymes are themselves metabolites, whose rate of formation and destruction varies with the conditions of growth. The effect of the substrate could then be due either to contributing the necessary organic molecules for the synthesis of the enzyme, or to affecting its stability (for instance by combining with it). The adaptive stimulation caused by the homologous substrate would only be one particular application of these principles.

Yudkin (86) formulated a "mass action theory" of enzyme production in which he assumed that, in all cases, the stimulation caused by the proper substrate does not result in the production of a new enzyme, but only increases the production of an enzyme otherwise formed in small amounts. He postulated also that in the cell the enzyme is in equilibrium with a precursor. Any substance combining with the active enzyme would then disturb the equilibrium and thus cause the production of more enzyme from the precursor; the adaptive stimulation by the homologous substrate could be explained on this ground. Yudkin has offered many facts in support of his theory; there are a few, however, which are perhaps in conflict with it. Both in the case of the organism which hydrolyses the capsular polysaccharide of Type III pneumococcus, and of the organism which decomposes creatinine, the addition of 1 to 2 per cent casein hydrolysate to media containing the specific substrates retards markedly the appearance of the homologous enzyme, although it does activate and increase growth. The final yield of enzyme is not decreased; only its rate of production is slower. It has been observed that the casein hydrolysate is more readily utilized than either the polysaccharide or creatinine and that its presence in the medium

retards the decomposition of the two latter substances. It would seem, therefore, that the formation of the specific enzyme depends not upon the presence of the homologous substrate in the medium, but upon its utilization by the metabolizing cell.

Wooldridge (82) pictures the cell surface as "comparatively loosely-knit structures composed largely of complex organic molecules, the latter orienting themselves in the surface as the result of the affinities exhibited between the various groupings of that molecule and those possessed by the adjacent molecules in the surface and the molecules in true solution on either side of the surface." In this way, a substrate present during the growth of a cell will tend to increase the concentration of enzymes available to act on that substrate. In some respects this hypothesis recalls the mechanism invoked by Breinl and Haurowitz (4) and by Mudd (58) to explain how an antigen determines the specific configuration of an antibody molecule (67); these authors suggested that during the synthesis of antibody globulin, the arrangement of amino acids is modified by the polar forces of the antigen in contact with the structure where the antibody is produced. It is worth repeating here that the formation of adaptive enzymes probably takes place at a time when new protoplasm is synthesized by the bacterial cell.

THE SPECIFICITY OF ADAPTIVE ENZYMES

Adaptive enzymes exhibit a remarkable specificity toward the substrates which have stimulated their formation. Karström has shown, for instance, that the adaptive enzymes of *Betacoccus arabinosaceus* can differentiate between different monosaccharides and polysaccharides (45, 48). Diehl has claimed that casein and gelatin can be differentiated by the adaptive enzymes of some proteolytic organisms, but his results need to be confirmed by more accurate methods (7, 52).

The enzymes which hydrolyze the capsular polysaccharides of pneumococci differentiate between polysaccharides which give rise to cross reactions in specific antisera; for instance, the polysaccharide of gum acacia which reacts in Type III pneumococcus antiserum, is not affected by the enzyme which hydrolyses the

Type III polysaccharide (12). Even more striking is the difference between enzymes attacking the polysaccharides of Type III and Type VIII pneumococcus (30, 39). Both of these substances are composed of glucose and glucuronic acid, in the ratio of 1:1 for Type III and 1:3 for Type VIII and exhibit cross reaction in immune sera. However, the bacterial enzymes developed against each one of the polysaccharides fail to attack the other; in other words, the enzymes are even more specific than the antibodies obtained by immunization of experimental animals with the capsular antigens of pneumococci (11, 69).

The specificity of the adaptive creatinine oxidases obtained from two bacterial cultures has been established by testing these enzymes against a number of substrates related to creatinine (13). It has been found for instance that the mere addition of a methyl or acetyl group to the creatinine molecule completely inhibits the enzymes; the removal of the methyl group in position 3 (leaving glycocyamidine), or its shift from position 3 to position 5 retards considerably the action of one of the bacterial enzymes and inhibits completely the other. The enzyme which converts creatine into creatinine exhibits a similar specificity (14).

APPLICATIONS OF MICROBIAL ADAPTIVE ENZYMES

Because of their specificity, adaptive enzymes have already found a place in the analysis of several biological and biochemical problems. Karström for instance observed that *Escherichia coli* adapts itself to the fermentation of maltose and lactose through the formation of the disaccharidases maltase and lactase (47); it is therefore likely that the direct fermentation of the disaccharides without preliminary hydrolysis which has been postulated by Willstätter in the case of certain yeasts, does not take place in bacterial processes. By using a strain of *Escherichia coli* which produces maltase, but not invertase, Karström (46) also established that, contrary to Weidenhagen's claim, maltose and sucrose are not hydrolyzed by the same alpha glucosidase.

F. H. Johnson (44), working with luminescent bacteria, observed a complete inhibition of respiration and luminescence by alpha-methylglucoside. This inhibition, however, is followed by

a sudden "escape" with complete recovery of both respiration and luminescence, which Johnson attributed to the production of an adaptive enzyme.

The mechanism of production of molecular hydrogen by *Escherichia coli*, and the possible intermediate production of formic acid, have been investigated by means of formic hydrogenlyase (61, 72, 73).

In the course of studies on renal function, it became necessary to develop a method for the quantitative estimation of the very small amounts of creatinine present in blood. The identification and analysis of creatinine in biological fluids have thus far depended on colorimetric methods which are so nonspecific, that many authors have denied the very presence of creatinine in circulating blood. With the help of specific adaptive enzymes, it has been demonstrated that creatinine is indeed present in blood plasma and in the erythrocytes. Quantitative studies of the amount of creatinine present in the blood and urine of normal and nephritic humans are being used in an analysis of renal function in health and disease (57).

The recently discovered adaptive anhydrase which converts creatine into creatinine, offers an interesting example of the enzymatic combination of an amino and a carboxyl group to form the CO—NH linkage; this reaction may be useful in the study of the metabolism of creatine (14).

A few years ago, an adaptive enzyme capable of hydrolysing the capsular polysaccharide of Type III pneumococcus was extracted in solution from the cells of a saprophytic bacterial species. Experiments with the enzyme afforded additional and final evidence as to the rôle played by the capsular polysaccharides in determining the serological specificity of pneumococci (12). It was also possible to protect experimental animals (mice, rabbits and monkeys) against large numbers of infective doses of Type III pneumococcus by injection of the soluble enzyme; this treatment, however, was entirely ineffective against pneumococci of other types (1, 29, 31). These results emphasize once more the rôle of the capsular polysaccharides in conditioning the virulence of encapsulated pneumococci (11).

All organic matter in nature eventually becomes the prey of microorganisms which break it down through the agency of their cellular enzymes; in fact there are usually found several species of microorganisms capable of attacking one same chemical entity and the enzymes through which different microbial species attack the same substrate vary in their mode of action. It is obvious therefore that one can find in the microbial world enzymes capable of performing almost every conceivable type of biochemical reaction, many of which are not known to take place elsewhere in the animal or plant kingdoms. In many cases, as we have seen, microorganisms adapt themselves to the performance of a given biochemical reaction by the production of specific enzymes. On account of their cellular origin these adaptive enzymes are capable of functioning under moderate chemical conditions (pH, temperature, etc.) and this property, together with their specificity render them ideal reagents for the analysis of biological problems. It is apparent, therefore, that the readiness with which microorganisms produce adaptive enzymes suggests a method which will yield an infinite number of specific "physiological" reagents.

It is also true, on the other hand, that the production of adaptive enzymes is a striking example, fairly well defined in biochemical terms, of adaptive response of the living cell to changes in the environment. A consideration of this phenomenon brings the bacteriologist back into the main channels of biological thought, to the biological problem *par excellence*, the problem of adaptation. The study of the mechanism whereby microorganisms produce those enzymes which appear as an adaptive response to the presence of the homologous substrates in the culture medium, bids fair to throw light on some of the reactions involved in biological adaptation.

SUMMARY AND CONCLUSIONS

The production of enzymes by microorganisms is influenced by different factors. Some bacterial species, for instance, give rise to variants endowed with new enzymatic properties; these are hereditary characters. Certain substances increase the yield

of a given enzyme by contributing the necessary organic or inorganic molecules for its synthesis, or by preventing its inactivation.

In other cases, the production of a given enzyme is greatly stimulated when the substrate which it attacks is a constituent of the culture medium. These "adaptive" enzymes appear and reach their maximum development during the growth of the first transfer of the culture in the specific medium; they fail to be formed as soon as the culture is again transferred to a medium not containing the specific substrate. Although the production of adaptive enzymes need not be associated with cellular multiplication, all evidence available indicates that it involves the synthesis of new protoplasm. It is suggested that the synthetic process is, so to speak, oriented or guided by the chemical structure of the substrate which thus determines the specificity of the enzyme.

Adaptive enzymes do in fact exhibit a remarkable specificity toward the substrates which have stimulated their production and they bid fair, therefore, to serve as useful tools in the analysis of many biological and biochemical problems.

REFERENCES

(1) AVERY, O. T., AND DUBOS, R. J. 1931 The protective action of a specific enzyme against Type III pneumococcus infection in mice. J. Exptl. Med., **54**, 73–89.

(2) BACH, D. 1936 L'évolution des déshydrogénases du *Proteus vulgaris*. Compt. rend. soc. biol., **122**, 1068–1070.

(3) BRAUN, H., AND WÖRDERHOFF, P. 1933 Ueber die oxydativen und reduzierenden Fermentwirkungen des Ruhrbazillus Flexner. Zentr. Bakt. Parasitenk, I Orig., **128**, 50–81.

(4) BREINL, F., AND HAUROWITZ, F. 1930 Chemische Untersuchung des Präzipitates aus Hämoglobin und Anti-Hämoglobin-Serum und Bemerkungen über die Natur der Antikörper. Z. physiol. Chem., **192**, 45–57.

(5) BRUNTON, T. L., AND MACFAYDEN, A. 1889 The ferment-action of bacteria. Proc. Roy. Soc. London, **B46**, 542–53.

(6) DESNUELLE, P. 1939 Dégradation anaérobie de la cystéine par *B. coli*. III. Spécificité optique de la cystéinase. Bull. soc. chim. Mém. Series 5, **6**, 1304–1306.

(7) DIEHL, H. S. 1919 The specificity of bacterial proteolytic enzymes and their formation. J. Infectious Diseases, **24**, 347–361.

(8) DIENERT, F. 1900 Sur la fermentation du galactose et sur l'accoutumance des levures à ce sucre. Ann. inst. Pasteur, **14**, 139–189.

(9) DUBOS, R. J. 1932 Factors affecting the yield of specific enzyme in cultures of the bacillus decomposing the capsular polysaccharide of Type III Pneumococcus. J. Expt. Med., **55**, 377–391.

(10) DUBOS, R. J. 1935 Studies on the mechanism of production of a specific bacterial enzyme which decomposes the capsular polysaccharide of Type III Pneumococcus. J. Exptl. Med., **62**, 259–269.

(11) DUBOS, R. J. 1939 Enzymatic analysis of the antigenic structure of pneumococci. Ergeb. Enzymforsch., **8**, 135–148.

(12) DUBOS, R. J., AND AVERY, O. T. 1931 Decomposition of the capsular polysaccharide of Pneumococcus Type III by a bacterial enzyme. J. Exptl. Med., **54**, 51–71.

(13) DUBOS, R. J., AND MILLER, B. F. 1937 The production of bacterial enzymes capable of decomposing creatinine. J. Biol. Chem., **121**, 429–445.

(14) DUBOS, R. J., AND MILLER, B. F. 1938 A bacterial enzyme which converts creatine into its anhydride creatinine. Proc. Soc. Exptl. Biol. Med., **39**, 65–66.

(15) DUCLAUX, E. 1883 Marche de la sécrétion des diastases. Duclaux, Encyclopedie chimique Frémy, Paris **9**, Section I, 190–192.

(16) DUCLAUX, E. 1899 Causes qui influent sur la sécrétion des diastases. Duclaux Traité de Microbiologie, Paris, **2**, 83–93.

(17) v. EULER, H., AND ASARNOJ, S. 1920 Zur Kenntnis der Enzymbildung bei *Aspergillus niger*. Fermentforschung, **3**, 318–329.

(18) v. EULER H., AND CRAMÉR, H. 1913 Untersuchungen über die chemische Zusammensetzung und Bildung der Enzyme. IX. Zur Kenntnis der Invertasebildung. Z. physiol. Chem., **88**, 430–444.

(19) v. EULER H., AND CRAMÉR, H. 1914 Zur Kenntnis der Invertasebildung in Hefe. Biochem. Z., **58**, 467–469.

(20) v. EULER, H., AND JANSSON, B. 1927 Über die Anpassung von frischen Kulturhefen an Galaktose. Z. physiol. Chem., **169**, 226–234.

(21) v. EULER, H., AND JOHANSSON, D. 1912 Untersuchungen über die chemische Zusammensetzung und Bildung der Enzyme. IV. Über die Anpassung einer Hefe an Galaktose. Z. physiol. Chem., **78**, 246–265.

(22) v. EULER, H., AND JOHANSSON, D. 1913 Untersuchungen über die chemische Zusammensetzung und Bildung der Enzyme. VIII. Über die gleichzeitige Veränderung des Gehaltes an Invertase und an Gärungsenzymen in der lebenden Hefe. Z. physiol. Chem., **84**, 97–108.

(23) v. EULER, H., LAURIN, I., AND PETTERSSON, A. 1921 Anpassung einer Oberhefe an das Gärsubstrat Galaktose. Biochem. Z., **114**, 277–291.

(24) v. EULER, H., AND LÖVGREN, T. 1925 Die durch Vorbehandlung hervorgerufene Gärfähigkeit frischer Hefe für Galaktose und die Konstanz dieser Eigenschaft. Z. physiol. Chem., **146**, 44–62.

(25) v. EULER, H., AND MEYER, H. 1912 Untersuchungen über die chemische Zusammensetzung und Bildung der Enzyme. V. Zur Kenntnis der Invertasebildung. Z. physiol. Chem., **79**, 274–300.

(26) v. EULER, H., AND NILSSON, R. 1925 Über die Galaktosevergärung durch Hefe nach Vorbehandlung mit dieser Zuckerart. Z. physiol. Chem., **143**, 89–107.

(27) FERMI, C. 1891 Weitere Untersuchungen über die tryptischen Enzyme der Mikroorganismen. Zentr. Bakt. Parasitenk., **10**, 401–408.

(28) FILDES, P. 1938 The production of indole by suspensions of *Bact. coli*. Biochem. J. **32**, 1600–1606.

(29) FRANCIS, T., JR., TERRELL, E. E., DUBOS, R. J., AND AVERY, O. T. 1934 Experimental Type III pneumococcus pneumonia in monkeys. II. Treatment with an enzyme which decomposes the specific capsular polysaccharide of Pneumococcus Type III. J. Exptl. Med., **59**, 641–668.

(30) GOEBEL, W. F. 1935 Chemo-immunological studies on the soluble specific substance of pneumococcus. II. The chemical basis for the immunological relationship between the capsular polysaccharides of Types III and VIII Pneumococcus. J. Biol. Chem., **110**, 391–398.

(31) GOODNER, K. G., DUBOS, R. J., AND AVERY, O. T. 1932 The action of a specific enzyme upon the dermal infection of rabbits with Type III Pneumococcus. J. Exptl. Med., **55**, 393–404.

(32) GORR, G., AND WAGNER, J. 1933 Über das Amidspaltungsvermögen der *Torula utilis*, eine Untersuchung über die Abhängigkeit pflanzlicher Enzymausbildung von der Stickstoffernährung. Biochem. Z., **266**, 96–101.

(33) GOULD, B. S., TYTELL, A. A., AND HUGHES, W. L. 1939 Studies in the biochemistry of Fusaria. Proc. Third Intern. Congr. Microbiology, New York, 230–232.

(34) HAINES, R. B. 1932 The influence of the medium on the production of bacterial gelatinase. Biochem. J., **26**, 323–336.

(35) HAINES, R. B. 1933 Further studies of the effect of the medium on the production of bacterial gelatinase. Biochem. J., **27**, 466–474.

(36) HALL, I. C. 1935 Metabolic "mutation" and colonial dissociation in the genus Bacterium. J. Bact., **29**, 13.

(37) HAPPOLD, F. C. 1939. The production of tryptophanase by bacteria. Proc. Third Intern. Congr. Microbiology, New York, 220–221.

(38) HEGARTY, C. P. 1939 Physiological youth as an important factor in adaptive enzyme formation. J. Bact., **37**, 145–152.

(39) HEIDELBERGER, M., KABAT, E. A., AND SHRIVASTAVA, D. L. 1937 A quantitative study of the cross reaction of Types III and VIII Pneumococci in horse and rabbit antisera. J. Exptl. Med., **65**, 487–496.

(40) HENRICI, A. T. 1928 Morphologic Variation and the Rate of Growth of Bacteria. C. C. Thomas, Baltimore.

(41) HERSHEY, A. D., AND BRONFENBRENNER, J. 1938 Factors limiting bacterial growth. III. Cell size and "physiologic youth" in Bacterium coli cultures. J. Gen. Physiol., **21**, 721–728.

(42) HOOGERHEIDE, J. C. AND WEIL, L. 1939. Studies on the metabolism and on the proteolytic enzymes of the strict anaerobes. Proc. Third Intern. Congr. Microbiology, New York, 216–217.

(43) JACOBY, M. 1917 Über Fermentbildung. Biochem. Z., **81**, 332–341.

(44) JOHNSON, F. H. 1938 Hexose oxidation by luminous bacteria. III. The escape of respiration and luminescence from inhibition by alpha methylglucoside, with a note on urethanes. J. Cellular Comp. Physiol., **12**, 281–294.

(45) KARSTRÖM, H. 1930 Über die Enzymbildung in Bakterien und über einigie physiologische Eigenschaften der untersuchten Bakterienarten. Thesis Helsingfors.

(46) KARSTRÖM, H. 1931 Zur Spezifität der α-Glucosidasen. Biochem. Z., **231**, 399–403.

(47) KARSTRÖM, H. 1932 Über die Laktase der Bakterien. Acta Chemica Fennica, **B5**, 44.

(48) KARSTRÖM, H. 1937–8 Enzymatische Adaptation bei Mikroorganismen. Ergeb. Enzymforsch., **7**, 350–376.

(49) KARSTRÖM, H. 1937 The problem of adaptation in bacteriology. Proc. Second Intern. Congr. Microbiology, London, 473–474.

(50) KENDALL, A. I., AND WALKER, A. W. 1915 Observations on the proteolytic enzyme of *Bacillus proteus*. Studies in bacterial metabolism, XL. J. Infectious Diseases, **17**, 442–453.

(51) KLUYVER, A. J., AND HOPPENBROUWERS, W. J. 1931 Ein merkwürdiges Gärungsbakterium: Lindner's *Termobacterium mobile*. Arch. Mikrobiol., **2**, 245–260.

(52) KOCHOLATY, W., AND WEIL, L. 1938 Enzymic adaptation in *Clostridium histolyticum*. Biochem. J., **32**, 1696–1701.

(53) LEWIS, I. M. 1934 Bacterial variation with special reference to behavior of some mutabile strains of colon bacteria in synthetic media. J. Bact. **28**, 619–638.

(54) MACFADYEN, A. 1892 A research into the nature and action of the enzymes produced by the bacteria. J. Anat. and Physiol., London, **26**, 409–429.

(55) MASSINI, R. 1907 Über einen in biologischer Beziehung interessanten Kolistamm (*Bacterium coli mutabile*). Ein Beitrag zur Variation bei Bakterien. Arch. Hyg. Bakt., **61**, 250–292.

(56) MERRILL, A. T., AND CLARK, W. M. 1928 Some conditions affecting the production of gelatinase by proteus bacteria. J. Bact., **15**, 267–296.

(57) MILLER, B. F., ALLISON, M. J. C., AND BAKER, Z. 1939 Studies on the metabolism of creatine and creatinine. J. Biol. Chem., **130**, 383–391.

(58) MUDD, S. 1932 A hypothetical mechanism of antibody formation. J. Immunol., **23**, 423–427.

(59) NEISSER, I. M. 1906 Ein Fall von Mutation nach de Vries bei Bakterien und andere Demonstrationen. Zentr. Bakt. Parasitenk., I, Ref. **38**, Beih., 98–102.

(60) ORDAL, E. J., AND HALVORSON, H. O. 1939 A comparison of hydrogen production from sugars and formic acid by normal and variant strains of *Escherichia coli*. J. Bact., **38**, 199–220.

(61) ORDAL, E. J. AND TSUCHIYA, H. M. 1939. The effect of induced variation on fermentation by *Escherichia communior*. Proc. Third Intern. Congr. Microbiology, New York, 217–219.

(62) PASSMORE, R., AND YUDKIN, J. 1937 The effect of carbohydrates and allied substances on urease production by *Proteus vulgaris*. Biochem. J., **31**, 318–322.

(63) PENFOLD, W. J. 1910 Variations of the fermentation properties of the *B. typhosus*. Brit. Med. J., **2**, 1672–1673.

(64) PENFOLD, W. J. 1911 Studies in bacterial variation. With special reference to the chemical functions of the members of the typhoid-coli group. J. Hyg., **11**, 30–67.

(65) QUASTEL, J. H. 1937 Bacterial enzyme formation as a function of the nutritional medium. Proc. Second Intern. Congr. Microbiology, London, 471–472.

(66) QUASTEL, J. H. 1937 Enzyme formation in Bacteria. Enzymologia, **2**, 37–42.

(67) RAHN, O. 1938 On the nature of adaptive enzymes. Growth, **2**, 363–367.

(68) RÉGNIER, J., LAMBIN, S., AND JUND, Y. 1938 Des variations morphologiques des bactéries en fonction de l'age de la culture. Essais sur le colibacille cultivé en bouillon nutritif. Compt. rend. soc. biol., **128**, 742–744.

(69) SICKLES, G. M., AND SHAW, M. 1935 A microörganism which decomposes the specific carbohydrate of Pneumococcus Type VIII. Proc. Soc. Exptl. Biol. Med., **32**, 857–858.

(70) SLATOR, A. 1908 Studies in fermentation. II. The mechanism of alcoholic fermentation. J. Chem. Soc., **93**, 217–242.

(71) STEPHENSON, M. 1937 Formic hydrogenlyase. Ergeb. Enzymforsch., **6**, 139–156.

(72) STEPHENSON, M., AND STICKLAND, L. H. 1932 Hydrogenlyases. Bacterial enzymes liberating molecular hydrogen. Biochem. J., **26**, 712–724.

(73) STEPHENSON, M., AND STICKLAND, L. H. 1933 Hydrogenlyases. III. Further experiments on the formation of formic hydrogenlyase by *Bact. coli*. Biochem. J., **27**, 1528–1532.

(74) STEPHENSON, M., AND YUDKIN, J. 1936 Galactozymase considered as an adaptive enzyme. Biochem. J., **30**, 506–514.

(75) VIRTANEN, A. I. 1934 On the enzymes of bacteria and bacterial metabolism. J. Bact., **28**, 447–460.

(76) VIRTANEN, A. I., KARSTRÖM, H., AND TURPEINEN, O. 1930 Über die Vergärung von Dioxyaceton. Z. physiol. Chem., **187**, 7–44.

(77) VIRTANEN, A. I., AND WINTER, A. O. 1928 Quantitative Enzymbestimmungen an Mikroorganismen. Über die Einwirkung einiger Faktoren auf den Katalasegehalt der Bakterien. Biochem. Z., **197**, 210–221.

(78) WAKSMAN, S. A. 1922 Enzymes of microörganisms. Abstracts Bact., **6**, 265–299.

(79) WINSLOW, C.-E. A., AND WALKER, H. H. 1939 The earlier phases of the bacterial culture cycle. Bact. Rev., **3**, 147–186.

(80) WOOLDRIDGE, W. R., AND GLASS, V. 1937 Variability in the activity of bacterial enzymes. II. Factors associated with viability and growth. Biochem. J., **31**, 526–531.

(81) WOOLDRIDGE, W. R., KNOX, R., AND GLASS, V. 1936 Variability in the activity of bacterial enzymes. I. The effect of the age of the culture. Biochem. J., **30**, 926–931.

(82) WOOLDRIDGE, W. R. 1937 The influence of substrate on the chemical potentialities of the cell. Proc. Second Intern. Congr. Microbiology, London, 474–475.

(83) WORTMANN, J. 1882 Untersuchungen über das diastatische Ferment der Bakterien. Z. physiol. Chem., **6**, 287–329.

(84) YUDKIN, J. 1932 Hydrogenlyases. II. Some factors concerned in the production of the enzymes. Biochem. J., **26**, 1859–1871.

(85) YUDKIN, J. 1937 Enzyme variation in micro-organisms. Proc. Second Intern. Congr. Microbiology, London, 476.

(86) YUDKIN, J. 1938 Enzyme variation in micro-organisms. Biol. Rev., Cambridge Phil. Soc., **13**, 93–106.

(87) ZIKES, H. 1914 Ein Beitrag zur Enzymbildung und deren Ursachen. Zentr. Bakt. Parasitenk., II, **41**, 246.